Research Note

The Response of *Tubifex tubifex* (Oligochaeta: Tubificidae) to a Second Infection with *Glaridacris catostomi* (Cestoidea: Caryophyllaeidae)

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ABSTRACT: The response of the aquatic oligochaete, *Tubifex tubifex*, to primary and secondary infection by the caryophyllaeid cestode, *Glaridacris catostomi*, was investigated by comparing rates of parasite mortality between the 2 infections. *Tubifex tubifex* that lost the first infection of *G. catostomi* became infected after second exposure to the cestode, but rate of parasite mortality did not increase. Over an 8-day period, both cumulative parasite mortality and percentage of hosts that lost their caryophyllaeid infection were greater for the first infection (90% and 59%) than for the second (46% and 2%). A cellular response, involving the encapsulation of metacestodes by host coelomocytes, accounted for part of the high caryophyllaeid mortality observed.

KEY WORDS: Cestoidea, Caryophyllidea, Tubificidae, metacestode.

Much of the literature on annelid immunity has involved the response of terrestrial earthworms to tissue grafts (Cooper, 1986), the coelomocytes active in the phagocytosis process (Stein et al., 1977), and characterization of antibacterial molecules (Valembois et al., 1986). Additionally, studies have been conducted on the encapsulation of parasitic organisms in annelids. Poinar and Thomas (1975) described the encapsulation of the nematode, *Rhabditis pellio*, in the coelom of the earthworm, *Aporrectodea trapezoides*, which involves the encasement of the parasite in host coelomocytes and formation of multiple capsules or "brown bodies." A similar reaction was observed by Calentine et al. (1970) in tubificid oligochaetes infected with caryophyllaeid cestodes. Host coelomocytes attached to the metacestodes, but no "brown bodies" developed. This cellular response against caryophyllaeids was reported for a number of tubificids including *Tubifex tubifex* Müller, 1774. This species was used in the present study to compare rates of parasite mortality between a first infection with *Glaridacris catostomi* Cooper, 1920, and a second infection with the same caryophyllaeid species.

Laboratory reared oligochaetes were experimentally infected by 24-hr exposure to embryonated cestode eggs in mud-free water (1,200–1,800 eggs per 25 ml of water), under continuous aeration, at a temperature range of 18–21°C. Cestode eggs were obtained from the laboratory of Robert Calentine, University of Wisconsin–River Falls. Immediately after exposure to cestode eggs, infected annelids were separated from uninfected ones. Infected annelids were identified by the presence of oncospheres in the coelom. All annelids used in experiments were maintained under aeration in 100-ml glass beakers containing initially sterile mud and fed a diet of Tetramin® flaked fish food. At various days postexposure (PE), wet mounts of infected annelids were examined microscopically at 100× and/or 430× with bright field or phase contrast optics and the number of cestodes present in the coelom counted.

In 2 separate trials, 1-wk-old *T. tubifex* were exposed for the first time to *G. catostomi*. The individuals that became infected were designated as group 1 (first infection). Approximately 2½-wk-old oligochaetes in group 1 that lost their cestodes (the cestodes died) were reexposed a second time to *G. catostomi*. The oligochaetes that became reinfected were designated as group 2 (second infection). At the same time as group 2, another group of approximately 2½-wk-old annelids, designated the control group, was exposed to *G. catostomi* for the first time. Data were combined from both trials and rates of parasite mortality were compared among all 3 groups.

Cumulative host and parasite mortality and percentage of hosts that lost their caryophyllaeid infection were compared among the 3 groups using R × C contingency tables with chi-square analysis. Student's *t*-test was used to compare group means at the various days PE. Differences in each of these analyses were considered significant at $P < 0.05$.
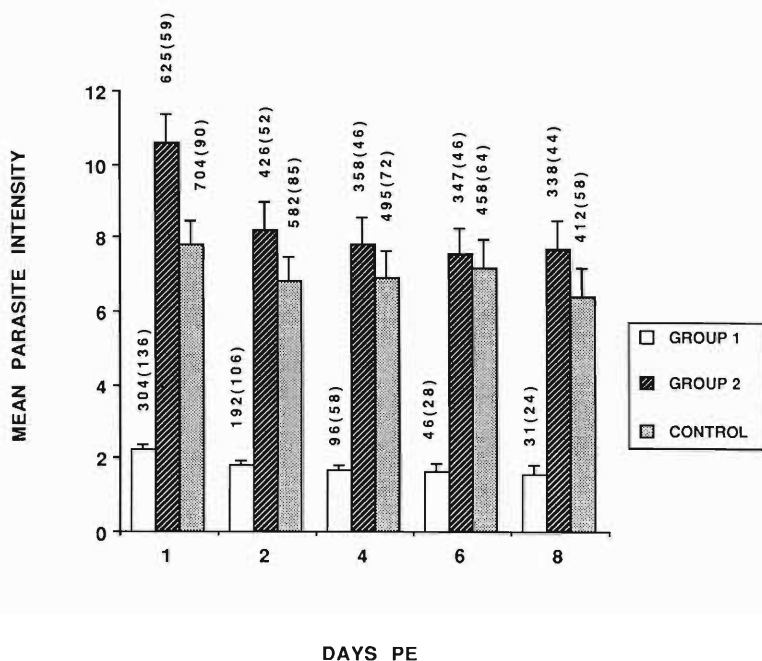
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Figure 1. Mean parasite intensities of *Glaridacris catostomi* in *Tubifex tubifex* for group 1 (first infection), group 2 (second infection), and a control group (same age as group 2). Bars represent the standard error of the mean. The numbers above each bar represent total number of parasites followed by total number of infected hosts in parentheses.

Overall, mean parasite intensity (Fig. 1) decreased over time for each group (e.g., from 2.23 at 1 day PE to 1.55 at 8 days PE in group 1, from 10.59 at 1 day PE to 7.68 at 8 days PE in group 2, and from 7.82 at 1 day PE to 6.38 at 8 days PE in the control group). Mean parasite intensities were higher in the second infection compared to the first infection. Only at 1 day PE was the mean parasite intensity for the second infection significantly larger than for the control group.

By 8 days PE, 90% of the cestodes were dead in group 1 hosts and 46% in group 2 (Fig. 2A). A significantly higher number of *G. catostomi*-infected *T. tubifex* lost their metacestodes in group 1 compared to group 2, 59% and 2%, respectively (Fig. 2C). Only 2% of control oligochaetes were no longer infected at 8 days PE (Fig. 2C). Host mortality was similar for all 3 groups (Fig. 2B).

Evidence of immunologic memory in annelids was provided by Cooper (1968) in his studies on tissue graft rejection in terrestrial earthworms. Secondary rejection of allografts and xenografts by earthworms was specific and enhanced (Cooper, 1968) and this memory was also transferrable with "sensitized" coelomocytes (Hostetter and Cooper, 1974). We attempted to measure the

potential for an adaptive immune response in aquatic oligochaetes by using a natural parasite of the annelid. However, on second exposure to *G. catostomi* in *T. tubifex* we did not find that the rate of parasite death increased. Thus, we concluded that the response exhibited by this host to the parasite is non-adaptive. Even after 35 days, percentage of hosts that lost their infection after second exposure was still lower than that for controls (5% vs. 18%). Parasite mortality was significantly higher in controls (73%) than in group 2 (61%) at 35 days PE. There appears to be no difference between susceptibility to infection on second exposure to *G. catostomi* for *T. tubifex* that previously lost the infection and those still infected with the parasite (see Calentine, 1967).

The high rate of parasite mortality seen in this study can be partially explained by a cellular response. We observed the encapsulation reaction in *G. catostomi*-infected *T. tubifex* that was reported by Calentine et al. (1970). Host coelomocytes attached to the metacestode and eventually encased it. Because of the infrequency of the response (observed 4 times), and because we observed the death of older and larger metacestodes
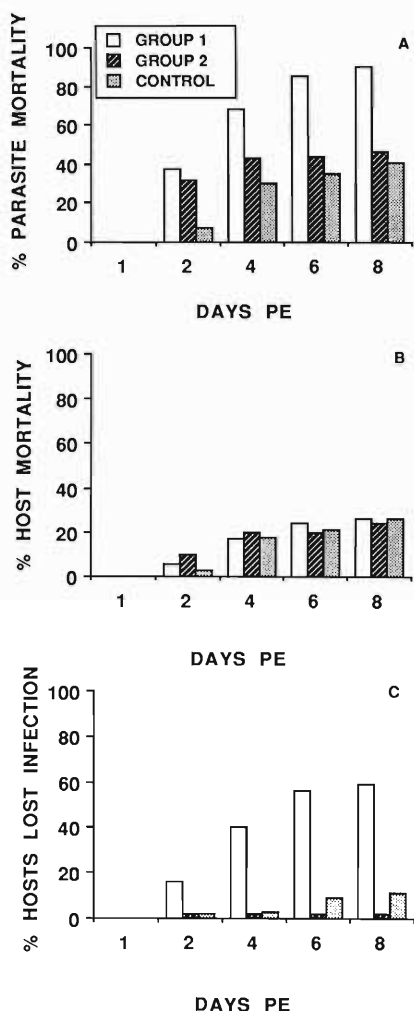
Figure 2. Percent cumulative parasite mortality (A), host mortality (B), and hosts that lost their caryophyllaeid infection (C) in *Glaridacris catostomi*-infected *Tubifex tubifex* for group 1 (first infection), group 2 (second infection), and a control group (same age as group 2).

without attachment of host cells, we believe that the encapsulation reaction alone is not the cause of the high parasite mortality seen in this study. Other mechanisms may be operating in this host–parasite system. The possible role of humoral immune components interacting with the coelomocyte population should be investigated.

Terrestrial earthworms possess humoral factors that are capable of agglutination and opsonization. A natural hemolysin of *Eisenia fetida andrei* shows lytic activity against sheep red blood cells (Roch, 1979) as well as antibacterial activity toward pathogenic soil bacteria (Lassègues et al., 1981; Valembois et al., 1982). Valembois et al. (1986) characterized the antibacterial molecules in this earthworm that are responsible for neutralization of pathogenic bacteria. Four proteins mediated hemolytic activity in the coelomic fluid but only 1 protein was involved in this process in the cocoon albumen. Stein and Cooper (1981) found that phagocytosis of yeast by neutrophils of *Lumbricus terrestris* was enhanced when the yeast were first washed with cell-free coelomic fluid. Whether the coelomic fluid of aquatic oligochaetes has similar immune capabilities is not known.

Age is an important component of the *G. catostomi–T. tubifex* association (Courtney and Christensen, 1988). Most infected tubificids do not become sexually mature (Sekutowicz, 1934; Kulakovskaya, 1962; Calentine, 1965, 1967) or the breeding period is delayed (Kennedy, 1969). Since young annelids are more susceptible to infection, the caryophyllaeid may be applying some mechanism that delays sexual maturity (e.g., liberating molecules that inhibit maturation of the gonads). A delay in host maturity could also be related to the depletion of host nutrients by the parasites. As a consequence of this, there is not enough energy available for reproductive growth. Additionally, the amount of space utilized by these cestodes may physically obstruct the development of the gonads. Thirty-day-old *T. tubifex* can have a body length of 25 mm or more (Kosiorek, 1974). The average length of an infective metacestode of *G. catostomi* is 1.2 mm (Calentine, 1967).

Equally important in the present study is the initial parasite intensity that can enhance the suitability of *T. tubifex* as a host (see Courtney and Christensen, 1987). When *T. tubifex* was subjected to a second exposure to *G. catostomi,* the initial parasite burden was higher and the percentage of hosts that lost their infection lower. If this phenomenon also occurs in tubificids in nature, then caryophyllaeids that parasitize previously infected hosts have a much higher probability of surviving to the infective stage. An increased susceptibility to infection can result in a higher parasite burden.

The increased susceptibility to caryophyllaeid infection could be a consequence of a reduction in the number of coelomocytes that occurred in the first infection. A reduced coelomocyte pop-

ulation in conjunction with depletion of humoral factors may account for a greater susceptibility to infection in group 2 compared to age-matched controls. In the snail, *Helix pomatia,* a decrease in the number of circulating blood cells was seen after injection of foreign particles. Additionally, the number of circulating blood cells can differ in snails of different ages with the number increasing in larger snails (Siminia, 1981). Further research is needed to determine the physiological differences between younger (more susceptible) annelids and older (less susceptible) annelids, and the immunocompetence of each.

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